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Design of an electrostatic phase shifting device for biological transmission electron microscopy

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ABSTRACT

I suggest an electrostatic phase plate designed to broaden the contrast transfer function of a transmission electron microscope operated close to Scherzer defocus primarily in the low resolution direction. At higher defocus the low frequency behavior is equal to that close to Scherzer defocus, but CTF-correction becomes necessary to extend image interpretation to higher resolution. One simple realization of the phase plate consists of two ring shaped electrodes symmetrically surrounding the central beam. Since no physical components come into contact with the central beam and charge on the electrodes is controlled by an external voltage supply, problems with uncontrolled charging are expected to be reduced.

1. Introduction

Zernike phase contrast has been recognized as a means of recording high resolution images with high contrast using a transmission electron microscope [1]. This imaging mode can be used to image typical phase objects such as unstained biological molecules or cryo sections of biological tissue. According to the original proposal reviewed in [2] and references therein the Zernike phase plate applies a phase shift of $\pi/2$ to all scattered electron beams outside a given scattering angle and an image is recorded at Gaussian focus or slight under-focus (below Scherzer defocus). Alternatively a phase shift of $-\pi/2$ is applied to the central beam using a Boersch-style [3] or a hole-free phase plate [4,5]. The resulting image will have an almost perfect phase contrast transfer function (PCTF) (close to 1) from a given lowest spatial frequency up to a maximum resolution determined by the wave length, the amount of defocus and the spherical aberration of the microscope. In the following I want to discuss a somewhat different way of thinking about what a phase plate is needed for in imaging weak phase specimens. A transmission electron microscope (TEM) operated at Scherzer or extended Scherzer defocus does an excellent job of transferring image information up to a maximum spatial frequency given by the electron wavelength λ and the spherical aberration coefficient C_s of the objective lens. Examples can be seen in the red curves of Fig. 1. Unfortunately the low frequency contrast transfer is too small to be able to image large scale biological structures, such as protein subunits, with high contrast. For example, as seen in the red curves of Fig. 1, the contrast transfer remains below 0.5 up to a spatial frequency of about 1 nm^{-1}.

Therefore one could argue that the main purpose of a phase plate should be to broaden the pass band at Scherzer or extended Scherzer defocus towards low spatial frequencies.

In principle this could be achieved by an additional phase shifting term similar to a sine-wave of appropriate wave length. Note again that this deviates from the usual conception about what a phase plate does, namely create a relatively constant phase difference between scattered and unscattered electrons. Here the phase plate just improves on the PCTF determined by a smart choice of defocus for a given C_s.

With such a phase plate the PCTF for weak phase objects is given by:

$$\mathrm{PCTF}(u) = \sin\left[\pi D \lambda u^2 + \frac{\pi}{2} C_s \lambda^3 u^4 - A \sin(k u)\right]$$

The additional term in the argument is just approximate and depends on what can be realized in practice.

Fig. 1a and b shows PCTFs with this extra phase shift and without for two choices of the parameters A and k.

A practical implementation of a phase plate that creates a phase shift similar to the one discussed above could make use of the phase shifting properties of thin films, electrostatic fields, magnetic

Abbreviations: TEM, transmission electron microscopy/microscope; PCTF, phase contrast transfer function.

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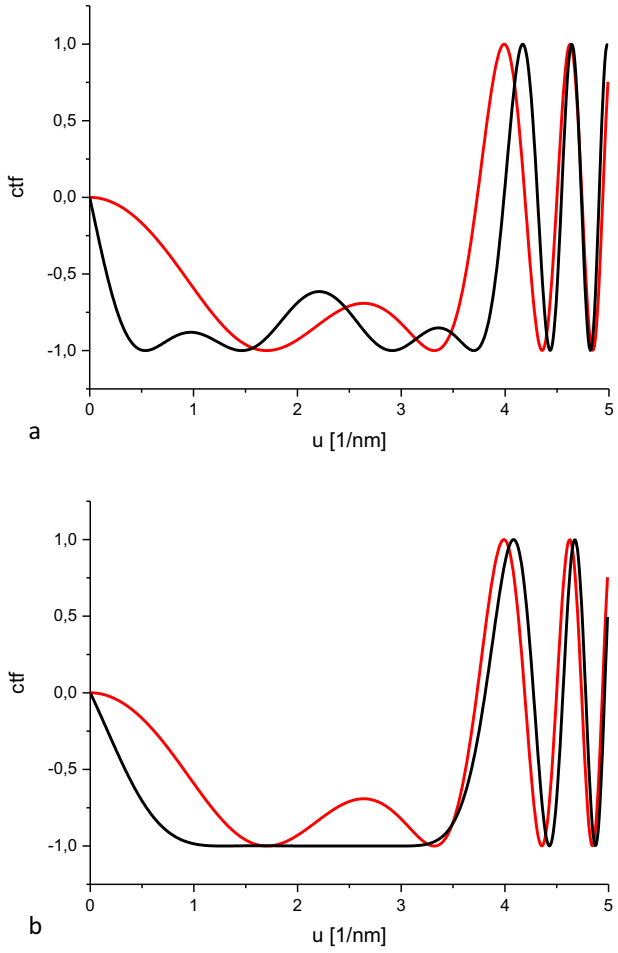

Fig. 1. PCTF for $C_s = 2$ mm; $\lambda = 0.0025$ nm; $D = -87$ nm; with the third term in the argument of the PCTF (black curves) and without the third term (red curves). The parameters are $A = \pi/2$ and $k = 2$ nm for (a) and $A = \pi/4$ and $k = 1.8$ nm for (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fields or standing light waves. An overview of different ideas can be found in [2].

2. Simulation of imaging with a phase plate consisting of two ring shaped electrodes

A relatively simple way of realizing an alternating phase-shift similar to what was discussed in the introduction is using two concentric rings with opposite charges. These electrodes could either be stable structures in themselves similar to the device discussed in [6] or they could be attached to the two faces of a very thin insulating or badly conducting film with a hole in the middle to let the central beam pass. The thickness of this film acting as a spacer should be such that it doesn't produce too much of a phase shift by itself. For example a 4 nm thick amorphous carbon film would produce a phase shift of about 18° in a 200 keV electron wave [7]. Alternatively the spacer could be the right thickness to cause a phase shift of approximately 2π. Carbon might be a good choice for separating the two electrodes since the electric current flowing through it due to the voltage difference between the electrodes might be sufficient to heat the electrodes and help keep them clean. A possible choice of material for the electrodes could be titanium which is carbon coated in the cleanest possible condition before mounting the assembly in the microscope. This combination of materials has been shown to have a very favorable charging behavior when used for a single side band aperture [8]. A suggestion for a design consisting of three layers that might possibly be produced with a focused ion beam (FIB) and then clamped together is shown in Fig. 2.

The phase as a function of spatial frequency produced by an appropriate choice of voltages on an idealized model of the two charged rings is shown in Fig. 3. The calculations leading to this figure are outlined in the supplement. In the given example the diameters of the two rings correspond to spatial frequencies of 1 and 0.25 nm^{-1}. For a 200 kV microscope with a focal length of about 4 mm the ring diameters would be 20 and 5 μm. The inner ring diameter is about a factor of 2 larger than that in the design discussed in [6] for a 100 kV instrument with a focal length of 5 mm. A smaller inner ring will improve the low resolution transfer but obviously the central beam should not come into contact with any structure, electrode or support film, in order to avoid uncontrolled charging. A practical compromise in this matter has to be found experimentally. However, it should be pointed out that the charge on both electrodes can be tuned to give the best phase contrast during operation of the microscope based on information from images recorded in real time. For comparison, a thin film phase plate with or without a central hole has no inbuilt mechanism for adapting to the charge that builds up due to the low angle portions of the electron beam.

As shown in Fig. 4 this phase plate combined with an underfocus value of 60 nm (slightly below Scherzer) produces a broad pass band in the PCTF for a 200 kV microscope with a C_s of 2 mm.

The behavior of the PCTF at very low spatial frequencies is comparable to that of the same microscope without phase plate at an under-focus setting of 500 nm as shown in Fig. 5. However, imaging with the proposed phase plate has the additional advantage of a single broad pass-band between about 40 and about 3 Å resolution rather than multiple pass-bands with alternating sign.

Image simulations show that an image produced with the phase plate at 60 nm underfocus (Fig. 6b) is a very faithful representation of the object (Fig. 6a). It has about the same contrast, estimated from the standard deviation, as the image recorded at 500 nm under-focus without a phase plate (Fig. 6f) and almost twice the contrast of images simulated without phase plate at under-focus values of 60, 71 (Scherzer) and 87 nm (extended Scherzer) (Fig. 6c–e). Again one has to consider that the total contrast is not the only parameter that affects visibility of proteins. Due to the oscillating nature of the PCTF at high defocus the image information in Fig. 6f is spread out over a larger area than in case of imaging with a phase plate (Fig. 6b). This should make it easier to detect and align particles in images recorded with a phase plate even if the contrast (measured by standard deviation) is not higher.

As shown in Fig. 7, the amount of underfocus can be varied without losing the good contrast transfer at low spatial frequencies. Obviously images recorded like that have to be CTF-corrected appropriately.

3. Materials and methods

Imaging simulations were carried out on the projection of a phantom representing a small protein hexamer in top view.

The electrostatic potential distribution inside trypsin inhibitor (Protein data Bank entry 4pti), a small protein with a diameter of about 2.5–3 nm, was calculated using Matlab-code written by Shang and Sigworth, which treats the molecule as a collection of neutral atoms as described in [9]. In this program each atom is assigned an atomic radius and a single potential value, obtained at the limit of low scattering angles from the parametrization of Kirkland [10].